

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

June, 1993 (1)

TABLE OF CONTENTS

	Page
Editor's comments	i
Oral History of William C. Reeves now available	ii

INVITED REPORT

Yellow fever in Kenya

Yellow fever outbreak, Baringo and Elgeyo Marakwet Districts, Rift Valley Province, Kenya, September 1992-February 1993 (A.A. Marfin, P.M. Tukei, N.N. Agata, E.G. Sanders, J.W. den Boer, I.P. Reiter, R.G. McLean, C.B. Cropp, P.S. Moore, D.J. Gubler) 1

Yellow fever in Kerio Valley, Rift Valley Province, Kenya: entomological investigation (P. reiter, R. Cordellier, J. Ouma, P.M. Tukei, G.B. Okelo, N. Agata, S.C. Cherogony, A.A. Marfin, C.B. Cropp, H.M. Savage, R.G. McLean, D.J. Gubler) 4

(Continued on next page)

PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE:

To exchange information on a timely basis. The recipients are those who study various aspects of arboviruses. The Exchange contains preliminary reports, summaries, observations, and comments submitted voluntarily by qualified agencies and individual investigators. The appearance in the Exchange of any information, data, opinions, or views does not constitute formal publication and should not be referred to in "Reference" sections of papers or included in lists of publications. The Exchange is not a "peer reviewed" publication; in fact, it is not a publication at all. Any reference to or quotation of any part of the Exchange must be authorized directly by the agency or person submitting the text.

Dengue activity in Malaysia between 1982-1992 (S.K. Lam)	7
Human sentinels for dengue surveillance in Yucatán, México (J.A. Farfán, M.A. Loroño, L.F. Flores, L.A. Manzano, E.P. Rosado)	9

VECTOR COMPETENCE AND VECTOR BIOLOGY

Biosystematics (R. Meiswinkel, H. Nevill, G.J. Venter, H. van Ark, D. Smith)	10
Epidemiology of <i>Culicoides</i> -transmitted viral diseases (G.J. Venter, K.G. Hermanides, E.M. Nevill, R. Meiswinkel, M. Swanepoel, M. Edwardes)	10
Other <i>Culicoides</i> research at Onderstepoort Veterinary Institute	11
Dry season survival of <i>Aedes aegypti</i> eggs in breeding sites in Mérida, Yucatán, México (J.A. Farfán, M.A. Loroño, L.F. Flores, L.A. Manzano, E.P. Rosado)	12

DEVELOPMENT AND APPLICATION OF TECHNIQUES

Eastern equine encephalitis virus antigen capture enzyme-linked immunosorbent assay (T.M. Brown, C.J. Mitchell, G.C. Smith, R.S. Nasci, D.J. Gubler, J.T. Roehrig)	14
--	----

MOLECULAR BIOLOGY AND MOLECULAR IMMUNOLOGY

Studies on variability of Toscana virus N protein (L. Nicoletti, M.G. Ciufolini, C. Giorgi, S. Mochi, A. Marchi)	16
Function of Japanese encephalitis virus nonstructural protein NS3 in RNA synthesis (T. Takegami [corresponded by S. Hotta])	19

EPIDEMICS AND SURVEILLANCE

Yellow fever outbreak in Maranhao State, Brazil, 1993 (A.P.A. Travassos da Rosa, S.G. Rodrigues, P.F.C. Vasconcelos, N. Degallier, M.A.P. Moraes, J.F.S. Travassos da Rosa, A.C.R. da Cruz)	20
Arbovirus surveillance in New Jersey, 1992 (S.I. Shahied, B.F. Taylor, W. Pizzuti)	21
A brief summary of surveillance for mosquito-borne encephalitis virus activity in California during 1992/93 ((R.W. Emmons)	23

Laboratory acquired infection with a new arenavirus 24
SPH 114.202- clinical and laboratory findings of a new human
disease. P.F.daC. Vasconcelos, A.P.A. Travassos da Rosa, S.G.
Rodrigues, R.B. Tesh, J.F.S. Travassos da Rosa, E.S. Travassos
da Rosa.

HANTAVIRUSES

Clinical features of haemorrhagic fever with renal syndrome in 25
Slovakia (O. Kozuch, A. Barakova, M. Labuda)

Icterus or ARF: leptospirosis or hantavirus disease 27
(J. Clements, P. McKenna, P. Matthys, C. McCaughey, G. Neild, S.
Hindrichsen, H. Leirs, G. van der Groen)

TRAVEL AND LEISURE

A Yank Down Under (C.H. Calisher) 32

Editor's comments

The size of this issue speaks for itself. From June, 1989 to January, 1993, issue sizes were (pages) 52, 88, 74, 82, 80, 105, 59, and 59. This issue contains a few more than 30 pages. If you no longer are interested in receiving the Arbovirus Information Exchange, or feel it no longer serves a useful purpose for you, the American Committee on Arthropod-borne Viruses should know this. The Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, continues to support this endeavor by providing enthusiasm for this newsletter and funding for the printings and mailings; they also should know this. Please write to me and let me know where you stand on this question and I will relay your thoughts.

On the other hand, if you consider this newsletter useful you should also consider contributing to it. Frequent, brief reports of your laboratory's activities, abstracts from papers you are submitting to a journal-- anything that would keep other virologists apprised of what is going on in the field-- these are what is desired.

Although funding for arbovirology has declined, both the prevalence of the diseases arboviruses cause and the interest in the viruses remain, albeit with a more molecular bent. It is imperative that arbovirologists remain an active interest group, else the studies of these viruses will become unrelated facets of the larger picture. The current experience among Native Americans in New Mexico is an excellent example of the need to maintain a critical mass of people interested in arboviruses and related viruses.

I look forward to hearing from you.

Charles H. Calisher, Ph.D.
AIDL, CSU Foothills Campus
Ft. Collins, CO 80523 (USA)

GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, submit only recent news and summaries of your work. PLEASE limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (single space the text); do not staple pages together; do not number pages.

NOTE: As noted in "PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE", which is found on the front page of each issue, you are encouraged to submit a brief summary of your work. The summary need not be in manuscript style, the results do not have to be definitive, you need not include tables (unless you want to). This is not a peer-reviewed publication. The intent is to communicate among ourselves and to let each other know what we are doing.

The next issue will likely be mailed in December, 1993 (probable deadline for submissions: November 15, 1993). There is nothing that requires you to wait until the last minute. If you have something to communicate in July, August, September, or October, please send it. Also, there is nothing that prevents you from submitting a report to every issue. There are no pages charges either but, then again, this is not a publication.

PLEASE !!!

Follow the directions for submitting reports. Double-spaced pages take twice as much space as single-spaced pages. Do not double-space or number pages. Single-space them and leave them unnumbered. Do not staple pages together.

Submission of reports

It does not matter where you send reports, so long as you send them. Mail to any of the three addresses below will reach the Editor, Arbovirus Information Exchange (Charles H. Calisher, Ph.D.):

c/o DVBID/NCID/CDCP
P.O. Box 2087
Ft. Collins, CO 80522 (USA)

or

2700 Granada Hills Dr.
Ft. Collins, CO 80525 (USA)
(Telephone: (303) 223-8586
(Fax: 303-225-1903)



REGIONAL ORAL HISTORY OFFICE
THE BANCROFT LIBRARY
(415) 642-7395

BERKELEY, CALIFORNIA 94720

4/93

PUBLIC HEALTH PIONEER WILLIAM C. REEVES RECORDS ORAL HISTORY

The story of pioneering work on two mosquito-borne diseases formerly epidemic in the western United States is preserved in the recently completed oral history of William Carlisle Reeves. The professor of epidemiology at the University of California School of Public Health for forty plus years has recorded his 686-page memoir, *Arbovirologist and Professor, UC Berkeley School of Public Health*, with the Regional Oral History Office of The Bancroft Library, University of California, Berkeley.

Described by his students as a dynamic, demanding, and imposing teacher, Professor Reeves received the University's Distinguished Teaching Award in 1981. He describes designing classroom lectures using the Socratic method to involve students in unraveling the mysteries of outbreaks of infectious diseases. The interviews also discuss his service as dean of the School of Public Health from 1967 to 1971, politically troubled years in the school's history. Drawing on his long association with the school, Professor Reeves provides an extensive history that includes the school's predecessor, the Department of Hygiene.

Reeves' research achievements have been honored by the major professional organizations in the fields of tropical medicine, medical entomology and infectious disease epidemiology. The principal legacies of this research are the balanced field-laboratory approach to elucidate the transmission cycles of arboviruses and the California Encephalitis Surveillance Program. Both of these continue to serve as models for other arbovirus research and control programs in the United States and many other countries of the world.

Dr. Reeves' early associate was Karl F. Meyer, director of the Hooper Foundation at the University of California, San Francisco, where Dr. Reeves was a research assistant from 1941-1949. His recollections of "K.F." supplement the Regional Oral History Office's volume on Dr. Meyer.

The Reeves oral history, conducted by Sally Smith Hughes, is available to readers at The Bancroft Library, UC Berkeley, and at UCLA's Department of Special Collections. To obtain bound, indexed copies at cost (\$112 plus \$4 shipping), make checks payable to: Regional Oral History Office, 486 Library, University of California, Berkeley 94720. (510) 642-7395.

NAME _____ No. Reeves vols. @ \$112 _____
ADDRESS _____ Postage @ \$4/vol _____
CITY _____ STATE _____ ZIP _____ AMOUNT ENCLOSED _____

Yellow Fever Outbreak, Baringo and Elgeyo Marakwet Districts, Rift Valley Province, Kenya, September 1992 - February 1993

Marfin AA¹, Tukei PM², Agata NN³, Sanders EG⁴, den Boer JW⁴, Reiter IP¹, McLean RG¹, Cropp CB¹, Moore PS¹, Gubler DJ¹

¹ Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; ² Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya; ³ Kenya Ministry of Health, Nairobi, Kenya; ⁴ World Health Organization, Nairobi, Kenya

Periodic outbreaks of yellow fever (YF) in East Africa have occurred since 1940 and the largest outbreak occurred in 1962 in Ethiopia, when an estimated 300,000 cases occurred.^{2,3} Despite relatively high anti-YF antibody prevalence in northern Kenya,^{4,5} outbreaks of YF have never been reported from this country. In the outbreak reported here, cases of hemorrhagic illness were first reported from Baringo and Elgeyo Marakwet Districts, Rift Valley Province, Kenya, in September, 1992. Subsequent serologic testing at the Division of Vector-Borne Infectious Diseases (CDC) showed that 17 persons had evidence of recent YF infection. A team from CDC joined investigators from the Kenyan Ministry of Health and WHO in February, 1993.

Case finding was started in Baringo and Elgeyo Marakwet Districts and expanded to Uasin Gishu, Laikipia, and Nakuru Districts. Cases of illness consistent with YF were persons with three of the following: jaundice, hemorrhage, encephalitis, renal failure, fever. Confirmed cases had this illness and serologic evidence of recent YF infection (specific IgM antibody by EIA or HI antibody titer $\geq 1:1280$ in a single sample, four-fold rise in HI antibody titer to YF virus in paired samples, or YF virus isolation). Serum and homogenates of liver were inoculated into neonatal mice and onto AP-61, C6/36 and Vero cell lines for virus isolation.

Fifty-three persons with hemorrhagic fever were identified from Baringo and Elgeyo Marakwet Districts (attack rate of 8.4 per 100,000). No cases were identified from the three adjacent districts. Of 53 persons with hemorrhagic illness, 21 (40%) were confirmed YF case-patients: 3 by virus isolation and 18 by serology. Confirmation was not possible for the remaining 32 persons. Earliest onset of illness was September 10, 1992 and the latest was February 18, 1993. Other reports of illness that occurred earlier and later are being investigated. Of the 53 clinical cases, 36, or 68% were males (Table 1). Of the 48 for whom definite age was known, 41, or 85%, were less than 40 years old (average age: 27.5 years; range: 8-65 years). Twenty-eight cases died resulting in a case fatality rate of 53%. YF incidence was not uniformly distributed across the two affected districts (Table 1). Of the 21 confirmed case-persons, all lived in remote woodlands and 19 lived between 1,500 and 2,100 meter elevation. No *Aedes aegypti* mosquito larvae were found at any of their homes.

This is the first documented outbreak of YF in Kenya. The sudden emergence of YF in Kenya is unusual because recent outbreaks have not been reported from adjacent countries. Medical record reviews suggested that a YF may have occurred 2-3 years before this outbreak. Unfortunately, the increasing prevalence of malaria in the region and the possibility of other hemorrhagic fever viruses make it difficult to identify YF outbreaks in the absence of serologic testing. Peridomestic transmission was unlikely because water was not stored and domestic vectors were not found at the homes of cases. Mosquito collections and subsequent virus isolations have demonstrated that the vectors in this outbreak were sylvatic species (see companion article). Persons in the affected areas had daily exposure to sylvatic mosquito species and the majority of YF cases were young males and persons living in the "bush." The potential for urban transmission must be considered because large cities are near the affected "bush" areas and introduction of YF to more populated areas could result in epidemic transmission by *Aedes aegypti*. Although there was no known urban transmission, surveillance should be established to detect early emergence of an urban epidemic. YF vaccination was the only possible intervention and the entire population of these two districts (> 700,000 persons) was vaccinated. Continuing enzootic transmission in the region can disperse YF beyond this vaccinated population. Although cases were only identified in Baringo and Elgeyo Marakwet Districts, other districts may possess similar ecological conditions that can support YF transmission to humans. There is an urgent need to identify potential sylvatic corridors which could conduct enzootic transmission into areas where the population has not been vaccinated.

REFERENCES

1. Monath TP; Craven RB; Adjukiewicz A; et al. *Am. J. Trop. Med. Hyg.*, 1980, 29(5):912-928.
2. Kirk R. *Ann. Trop. Med.*, 1941, 35:67-73.
3. Serie C; Lindrec A; Poirier A; et al. *Bull. WHO*, 1968, 38:835-841.
4. Henderson BE; Metselaar D; Cahill K; et al. *Bull WHO*, 1968, 38:229-237.
5. Henderson BE; Metselaar D; Kirya GB; Timms GL. *Bull. WHO*, 1970, 42:787-795.

Table 1. Number of clinical hemorrhagic yellow fever cases by age and sex, Baringo and Elgeyo Marakwet Districts, Kenya, 1992-93.

<u>Characteristic</u>	<u>Number</u>	<u>Attack Rate¹</u>
Total cases	53	8.4
Deaths (Case Fatality Rate)	28 (53%)	---
Sex,		
Male (%)	36 (68%)	---
Female (%)	17 (32%)	---
Age intervals,		
0-9 years (%)	1 (2%)	0.4
10-19 years (%)	17 (32%)	10.4
20-39 years (%)	23 (43%)	13.5
40-59 years (%)	4 (8%)	6.0
60+ years (%)	3 (6%)	12.5
Adult-Unspecified age (%)	5 (9%)	---
Residence,		
Baringo District		
Tenges Division	28	137.1
Kabarnet Division	11	20.6
Ravine Division	6	7.8
Other divisions	3	1.5
Total for district	48	13.3
Elgeyo Marakwet District		
Southern Division	5	16.7
Other divisions	0	0
Total for district	5	1.7

¹ Rates per 100,000 population.

Yellow Fever in Kerio Valley, Rift Valley Province, Kenya: entomological investigation

CDC recently collaborated with the Government of Kenya and the World Health Organization in an investigation of an outbreak of Yellow Fever (YF) in Elgeyo Marakwet and Baringo Districts, Rift Valley Province, Kenya. The outbreak was officially reported in late September 1992 and peaked in January, 1993. The last cases were reported in late February. Most confirmed cases lived in wooded areas between 1500m and 1920m altitude (mean 1737m; $n = 19$) in the southern Kerio Valley (ca. 0°7' N to 0°35' N; 35°34' W to 35°48' W), but a number were also reported from the eastern flank of the Tugen Hills, particularly towards the end of the outbreak.

There is no record of epidemic transmission of YF in Kenya, but large epidemics have occurred in Ethiopia and the Sudan, and the disease is endemic in Uganda. A disease fitting the description of YF occurred to the north of the present outbreak area in 1991 (51 deaths reported), indicating a possible southward progression of ca. 100 km per year.

The ecology of the south Kerio Valley area is complex, ranging from semi-arid thorn scrub and acacia savannah below 1200m to pine forest at 2400m. Monkeys of various species are found throughout the area.

We made human bait collections (mainly from 16.00h to 19.30h, but also from 08.30 to 12.00h on some days) in five study areas incorporating three distinct biotopes: (i) non-thorny bush/woodland, with some larger trees, (especially along watercourses), associated with the majority of confirmed cases; (ii) discontinuous gallery woodland bordering tributaries of the Kerio River on the semi-arid floor of the valley, an area reported to have been associated with the earliest (unconfirmed) cases; (iii) banana patches and thorn scrub, also on the floor of the valley, and also possibly associated with early (unconfirmed) cases. In a 19-day period in March, we collected 2700 mosquitoes, which were divided between CDC Fort Collins and the Kenya Medical Research Institute. Results reported here are from the CDC material only (115 pools, 1481 mosquitoes tested).

In the non-thorny bush/woodland biotope, *Aedes (Stegomyia) africanus* was by far the most commonly collected mosquito. At some sites there were significant numbers of *Aedes (Finlaya) ingrami* and *Ae. (S.) keniensis*. Simultaneous collections at ground level and on platforms in trees, 6m above the ground, indicated that whereas *Ae. africanus* fed both at canopy and at ground level, *Ae. ingrami* and *Ae. keniensis* only fed at ground level.

In the discontinuous gallery woodland the number of mosquitoes captured was very small, but included *Ae. (S.) luteocephalus*, *Ae. (S.) metallicus*, *Ae. (Aedimorphus) vittatus* and *Ae. (S.) aegypti*. *Ae. aegypti* was of the forest form, not associated with human dwellings and not considered to be an important vector because of its preference for non-primate hosts. The other species are implicated as YF vectors in semi-arid or arid regions of the continent. *Ae. africanus* and *Ae. keniensis* were not encountered.

The third biotope, banana patches and thorn scrub, was investigated because Heisch (1950) reported that *Ae. (S.) simpsoni* s.l. was common in the thorn scrub but not in the small banana patches along the foot of the Elgeyo Marakwet escarpment. We confirmed Heisch's findings: *Aedes (S.) bromeliae*, a member of the *Ae. (S.) simpsoni* group and an important YF vector, appeared common in the thorn scrub, but was not collected in banana orchards.

Water storage is not practiced by the peoples of the area (Tugen, Elgeyo and Marakwet), so domestic *Ae. aegypti* were essentially absent.

To date, we have obtained three YF isolates — one from a pool of *Ae. africanus* (5 mosquitoes) and two from pools of *Ae. keniensis* (3 mosquitoes each) collected at a separate site. Both sites were at the edge of streams in the non-thorny bush/woodland biotope, and were associated with a (confirmed) YF case with date of onset in the previous month; both cases were women who had visited these sites daily to collect water. Other virus assays are pending.

Ae. africanus is an important vector of YF in many parts of Africa, extending from rain forest to the fringes of the Sudan savannah. In the forest it is principally a canopy species, but it bites at ground level at the forest edge, and, as we observed, at both canopy and ground level in riverine forest (WHO, 1986). The abundance of this species in the biotope associated with the majority of cases, coupled with the isolation of virus, indicates that this may have been the principal vector during the outbreak.

Ae. keniensis was first recognized as a separate species by van Someren (1946), but no mention has been made in the literature since then, and to our knowledge this is the first time that it has been subjected to virus assays. Nothing is known about its distribution, bionomics or transmission potential, but as a *Stegomyia* species it is likely that it can function effectively as a YF vector. The observation that it may prefer biting at ground level could augment its significance in transmission to humans.

The entomologic data indicate that this was a purely sylvatic outbreak, with human cases directly linked to the epizootic via species such as *Ae. africanus* and *Ae. keniensis*. The epidemiologic data — scattered distribution of cases, rarity of multiple cases within homesteads, age profile, sex distribution, and other features — fully corroborate this interpretation (see accompanying report).

More than 720,000 inhabitants have been vaccinated, but this will not affect enzootic transmission, and the monkey population is adequate to support a continuing sylvatic cycle. An important but unanswered question, therefore, is whether the epizootic could disperse beyond the vaccination area. There is an urgent need to identify potential sylvatic corridors which could conduct enzootic transmission away from the Kerio Valley region into areas where the human population has not been vaccinated. We are attempting to approach this problem using satellite imagery and Geographic Information Systems (GIS).

After our investigations in the outbreak area, we made a quick series of larval surveys and afternoon human bait collections in urban and rural domestic settings in heavily populated areas within a 100-150 km radius of the outbreak area. Two ethnic groups inhabit the areas, the Luya (Kakamega and the surrounding countryside), and the Luo (the city of Kisumu, the country around Maseno and country around Ahero, on the Kano Plain). Both groups practice water storage. Among the Luya, and the Luo around Ahero, we did not find *Ae. aegypti* in the water storage jars, perhaps because of the excellent supply of clean water in the areas concerned. By contrast, indices were high the Maseno area, where water was taken from streams, and in some parts of Kisumu (we await mosquito identifications from Kenya). We did not collect *Ae. bromeliae*, but stress that this was a rapid survey in a season that was probably not optimal for adults of this species. We conclude that introduction of YF virus to these heavily populated areas, whether via the enzootic cycle or by direct introduction in an infected traveller, could result in a major epidemic transmitted by peri-domestic mosquitoes.

Heisch, R. B. 1950. Survey of the Kerio Valley. *East African Medical Journal* pps. 233-242.

van Someren, E. C. C. 1946. Ethiopian Culicidae: notes and descriptions of some new species and hitherto unknown larvae and pupae (Diptera). *Trans. R. Ent. Soc London* 96:109-124

WHO 1986. Prevention and control of yellow fever in Africa. WHO Geneva. 94 pps.

P. Reiter
R. Cordellier
J. Ouma
P.M. Tukei
G.B. Okelo
N. Agata
S.C. Cherogony
A. A. Marfin
C. B. Cropp
H. M. Savage
R. G. McLean
D. J. Gubler

DENGUE ACTIVITY IN MALAYSIA BETWEEN 1982-1992

S.K. LAM

WHO Collaborating Centre for Dengue Fever/Dengue Haemorrhagic Fever,
Department of Medical Microbiology, Faculty of Medicine,
University of Malaya, 59100 Kuala Lumpur, Malaysia.

Dengue is endemic in Malaysia and all four dengue serotypes are known to be circulating in any one year (Table 1). In 1992, the predominant strain was dengue 3, a strain which was responsible for the outbreaks in 1985 and 1986. It is noted that one dengue strain predominates during the peak season and can account for up to 90% of all virus strains isolated. Another point of note is the persistence of the dominant virus for at least two to three years before it becomes replaced by another serotype.

Table 2 shows the dengue activity in Malaysia between 1982-1992. There were 29,487 notified cases of which 24,723 were DF and 4,764 were DHF, giving a DF:DHF ratio of 5.2:1. Based on 6,970 laboratory confirmed dengue cases by the WHO Centre, the male to female ratio was 1.2:1 and the ratio among the 3 major ethnic groups (Chinese:Malays:Indians) was 5.1:2.9:1. This was reflected every year during this period.

There were 184 deaths during this period, giving rise to a case fatality rate (CFR) of 0.62%. The predominant virus strains responsible for the outbreaks are presented in Table 2 and summarized in Table 3. The CFR was highest when the predominant strain was dengue 3 (0.77%), followed by dengue 2 (0.54%) and dengue 1 (0.35%). The overall CFR for this period was 0.60%. It is apparent that in Malaysia at least, dengue 3 is responsible for many of the severe outbreaks in the last 11 years.

Since the predominant virus strain persists for at least 2 years, the Centre predicts that dengue 3 will be the major virus in 1993 and that the season will be severe. The dengue season in Malaysia usually starts in May each year and peaks in August. Figures up till the end of May this year have confirmed this prediction and the Centre has identified dengue 3 virus in over 90% of its isolates so far, including many from severe DHF/DSS. The Ministry of Health has been placed on the alert to avert a worsening dengue situation.

Table 1 CIRCULATING VIRUS SEROTYPES (1985-1992)

1985	*DEN 3	DEN 4	DEN 2	
1986	DEN 3	DEN 1	DEN 4	DEN 2
1987	DEN 1	DEN 3	DEN 2	DEN 4
1988	DEN 1	DEN 2	DEN 4	DEN 3
1989	DEN 2	DEN 1	DEN 3	DEN 4
1990	DEN 2	DEN 1	DEN 3	DEN 4
1991	DEN 2	DEN 3	DEN 1	DEN 4
1992	DEN 3	DEN 2	DEN 4	DEN 1

Table 2

CASE FATALITY RATE FOR DENGUE IN MALAYSIA
BETWEEN 1982-1992

Year	No. of Death	No. of Cases	CFR%	Virus
1982	36	3126	1.15	D3
1983	10	790	1.27	NA
1984	5	697	0.72	NA
1985	11	354	3.11	D3
1986	9	1399	0.64	D3
1987	9	2002	0.45	D1
1988	3	1428	0.21	D1
1989	16	2563	0.62	D2
1990	21	4879	0.43	D2
1991	39	6621	0.59	D2
1992	25	5628	0.44	D3
	184	29487	0.62	

NA - Not available

Table 3

CASE FATALITY RATE BASED ON MAJOR
DENGUE SEROTYPES

Major serotype	Year	Death	Total DF+DHF	CFR%
Dengue 1	1987, 1988	12	3,430	0.35
Dengue 2	1989, 1990 1991	76	14,063	0.54
Dengue 3	1982, 1985 1986, 1992	81	10,507	0.77
Total	1982-1992	169	28,000	0.60

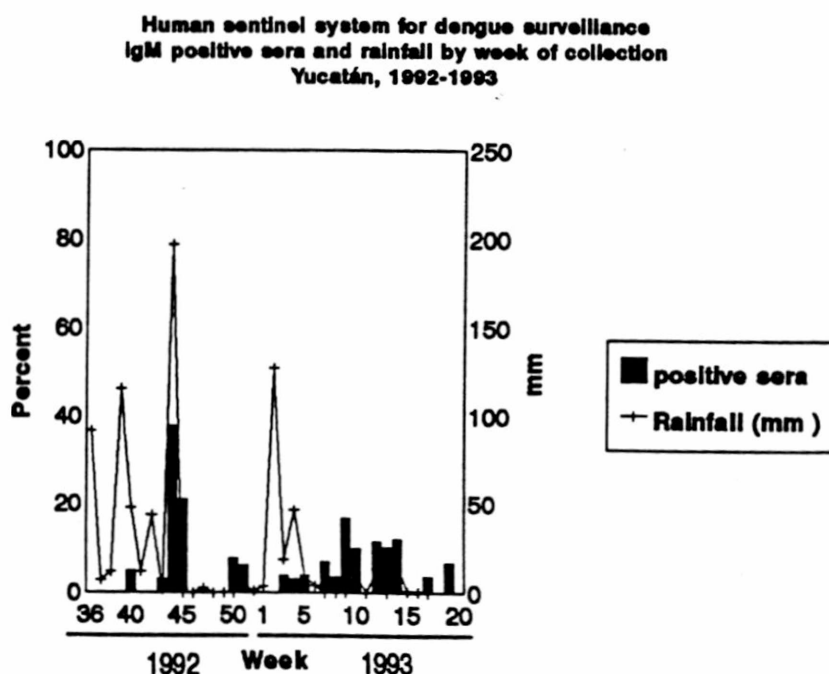
Farfán, JA; Loroño, MA; Flores, LF; Manzano, LA and Rosado, EP.

Human sentinels for dengue surveillance in Yucatán, México.

Dengue surveillance is useful because early case detection enable to set up interventions aimed to reduce the morbidity. The interest of physicians and health authorities on dengue surveillance decrease notably during the seasons when dengue transmission decreases. The interest and actions oriented to dengue control reappear when the epidemic has reached its peak. Surveillance is difficult because physicians tend to relay too much in clinical diagnosis, and also do not want to produce discomfort in the patients requiring blood samples from them. Dengue infection is subclinical in up to 65 to 70 per cent of those who have contact with the virus. IgM last up to one to one and a half months after the contact with the virus. A surveillance system is been tested using human sentinels and taking advantage of the existing infrastructure to collect sera.

Two of the main public hospitals are in the system. Twenty per cent of the sera collected in the hospitals for routine testing is collected randomly. Samples are taken from one of the hospitals monday and tuesday and from the other in the next two days. The sera is tested with IgM-capture ELISA. Sampling began on September 1992 and small peaks have been found (Fig. 1). Raining season is about to begin and we look forward to monitor through this system, the prevalence of infection in the next future.

Figure 1



CULICOIDES RESEARCH AT ONDERSTEEPOORT VETERINARY INSTITUTE

Private Bag X5, Onderstepoort 0110, Republic of South Africa

Biosystematics

R. Meiswinkel, H. Nevill, G.J. Venter, H. van Ark and D. Smith

Studies are being undertaken to revise the taxonomy of the bloodsucking genus *Culicoides* using both the adult and immature stages, to unravel the biology of as many species as possible and to apply this knowledge to clarifying the epidemiology of such arboviral diseases as African horsesickness. Fiedler (1951) recorded 22 species of *Culicoides* from South Africa; the Onderstepoort collection now includes approximately 110 species of which 35% are new to science. The growth of this species list has continued unabated for the last 8 years, an average of 5 species being discovered annually.

It has been found that 10 species (7 new and all of the subgenus *Avaritia*) breed exclusively in the dung of large mammals such as the elephant, rhinoceros, buffalo, wildebeest and zebra and that some of these *Culicoides* will 'cross over' and utilize the dung of domesticates especially cattle and horse. As all these species are closely related to the world-famous *C. imicola*, a known vector of African horsesickness and bluetongue, this close association between a midge, an animal and its dung, carries important implications for virus transmission and is being continuously researched. Simultaneously the pupal stages of nearly all of these new *Avaritia* species have been retrieved from various dung types and found to possess remarkable distinguishing features that have contributed strongly to clarifying the taxonomy of the difficult subgenus *Avaritia*.

As little is known about the epidemiology of African horsesickness a year-long survey has been initiated in the Kruger National Park to establish which *Culicoides* are closely associated with the plains zebra, the suspected reservoir host of African horsesickness. *C. sp. # 107*, a new species closely related to *C. imicola*, has been found to be strongly associated with zebra. This KNP study is being conducted in parallel with an AHS antibody survey of zebras darted in the same areas and led by Dr. B.J.H. Barnard of the Virology Division, Onderstepoort.

A 2-week *Culicoides* survey was conducted on sheep, cattle and horse farms in the eastern Orange Free State. Quite unexpectedly it was found that *C. imicola* comprised only 1,2% of all midges caught while *C. bolitinos*, which breeds in cattle dung, made up 50% of catches. As bluetongue is endemic in this part of the OFS we may now, for the first time, have found a species other than *C. imicola* that transmits this disease.

Mr. Alan Dyce, a research fellow of the CSIRO, Australia, spent 3 months at Onderstepoort on a project revising the world species of the Schultzei group (subgenus *Remmia*) containing important biters of stock in the more arid regions of Africa, the Near and Far East and Australia. Collections on loan from 30 countries revealed that at least 14 species constitute the group and not 6 as previously thought. Much work remains to be done on this important but misunderstood subgenus.

Epidemiology of *Culicoides*-transmitted viral diseases

G.J. Venter, K.G. Hermanides, E.M. Nevill, R. Meiswinkel, M. Swanepoel and M. Edwardes

Studies are continuing to determine which *Culicoides* species transmit African horsesickness, bluetongue and other arboviruses to stock in South Africa, the source and limits of infection and the conditions required for disease transmission to take place.

***Culicoides* distribution:** In a 3 year countrywide survey of the *Culicoides* species associated with stock, 50 of the 110 *Culicoides* species found in South Africa, were collected. The more abundant and widespread species, which have the potential to be vectors, are *C. imicola*, the *C. schultzei* group, *C. zuluensis*, *C. pycnostictus*, *C. leucostictus*, *C. bedfordi*, *C. magnus*, *C. rarus*, *C. gulbenkiani*, *C. similis* and *C. bolitinos*. *Culicoides imicola* accounted for 71,4% of the species collected. This species has a wide distribution and was especially abundant

in the warm, frost-free summer rainfall areas. As *C. imicola* is relatively uncommon in warm/dry and cool/wet areas this species cannot be regarded as the only potential vector of bluetongue. The most abundant species in the latter areas were, respectively, the *C. schultzei* group and *C. zuluensis*. Both these species have a wide distribution and can therefore also be potential vectors in other areas.

Blood-meal identification: To date 19 *Culicoides* species have been shown to have fed on livestock (cattle 18, horse 9, sheep 9, chicken 5). *C. imicola*, *C. zuluensis*, *C. magnus*, *C. bolitinos*, *C. gulbenkiani*, *C. schultzei* group and *C. engubandei* fed almost solely on mammals while *C. leucostictus* and *C. pycnostictus* fed mostly on birds and will therefore be less important as potential vectors of African horsesickness and bluetongue.

Arbovirus distribution: By means of questionnaires to veterinarians and stock inspectors, the geographical distribution and seasonal abundance of African horsesickness, bluetongue and three-day stiffness, was determined. Bluetongue and three-day stiffness are widely distributed in South Africa and occur regularly throughout most of the country. African horsesickness has a more limited distribution and outbreaks tend to be more common in the warmer parts of Transvaal, Northern Natal and Eastern Cape.

African horsesickness sentinels: In collaboration with the Directorate of Animal Health, sera from donkeys throughout the country are being tested for African horsesickness antibodies by means of an ELISA test. This will eventually provide us with more reliable data on the distribution and possible movement of this disease.

Report from Centro de Investigaciones Regionales "Dr. Hideyo Noguchi" de la Universidad Autónoma de Yucatán. Ap. postal 2-1297, Mérida, Yucatán, México. c.p. 97240.

Farfán, JA; Loroño, MA; Flores, LF; Manzano, LA and Rosado, EP.

Dry season survival of *Aedes aegypti* eggs in breeding sites in Mérida, Yucatán, México.

The *Aedes aegypti* eggs can remain viable during dry periods. When raining seasons begin, eggs hatch and the population of adults increase rapidly. The amount of mosquitos populations depends in part on the protection that the microenvironment provide to eggs. In order to see what kind of container gives better protection to *A. aegypti* eggs, it was placed bags with eggs in various common containers. Eggs of *A. aegypti* were obtained from females collected in Mérida biotopes. Batches of 100 eggs were placed in bags of fine-mill silk in order to protect them against ants. Five bags were placed in each container in shadowed areas. One set of five bags was kept in the laboratory as control. One bag was collected from each container in the field and one from the laboratory after periods of 2, 4, 6, 8 and 10 weeks. The eggs were counted in the laboratory and placed in containers with water in order let them hatch. The pupae produced were removed and counted to get the proportion of pupae obtained from each lot and the values analyzed by linear regression. It was studied the presence of parallelism among the slope of the control against the slopes from each container. Table 1 shows the data obtained applying the regression and hypothesis tests.

Table 1
Intercept and slope values from the regression equations with the data obtained in control and containers.

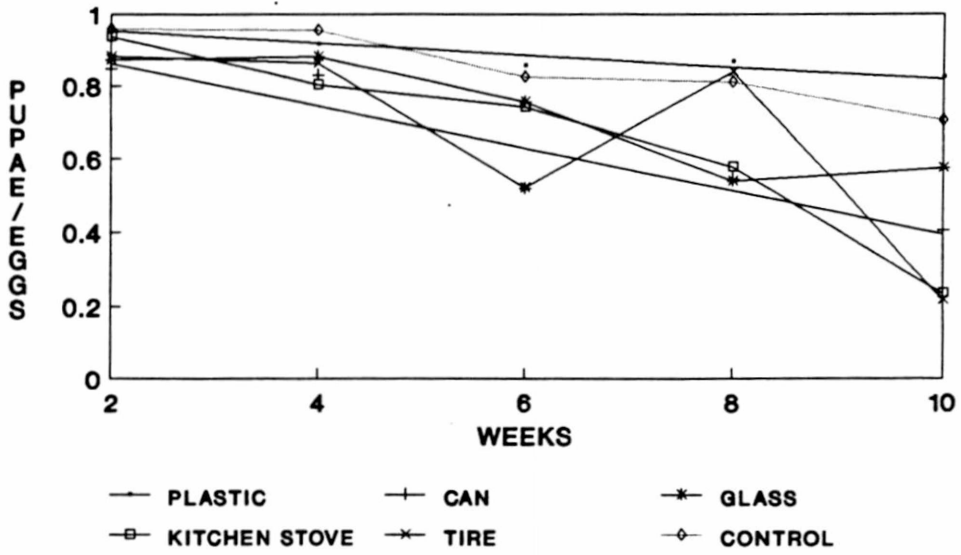
	B_0	B_1	t^*	p
Control	1.0450	-0.0325	----	
Plastic	0.9855	-0.0168	-2.47	< 0.05
Can	0.9784	-0.0583	1.83	NS
Glass	1.0053	-0.0469	1.11	NS
Kitchen stove	1.1472	-0.0815	3.16	< 0.05
Tire	1.0685	-0.0675	0.96	NS

* t Student values obtained comparing the slope in control vs. the slope in each container.

No significative statistical difference was obtained among the slope of plastic, can, tire and control, indicating that those breeding sites protected to eggs. The slope obtained with the data from the plastic container was closer to the horizontal than the slope in the control, that indicated that plastic gave better protection than control. The slope obtained with the data from the discarded kitchen stove was less than that in the control, suggesting less protection on the eggs (Figure 1).

Figure 1

Proportion of pupae obtained from the
eggs placed in different containers,
by week of collection



EASTERN EQUINE ENCEPHALITIS VIRUS ANTIGEN CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY

T.M. Brown, C.J. Mitchell, G.C. Smith, R.S. Nasci,
D.J. Gubler, J.T. Roehrig

Division of Vector-Borne Infectious Diseases, National Center for
Infectious Diseases, Centers for Disease Control and Prevention,
P.O. Box 2087, Fort Collins, CO 80522

An antigen capture enzyme-linked immunosorbent assay (ELISA) for the alphavirus, eastern equine encephalitis (EEE) virus has been developed. The general ELISA followed the design developed for St. Louis encephalitis virus (1,2) and was similar to previously designed capture ELISAs for EEE virus (3,4,5,6). The main modification in this technique was the use of a commercially prepared peroxidase (POD) conjugate of the North American EEE virus specific murine monoclonal antibody (Mab), 1B5C-3 (7) as detector of captured antigen. This Mab reacts with the E1 glycoprotein.

Virus antigen was captured in 96-well Immulon II microtiter plates previously sensitized with a 1:1000 dilution of a polyclonal anti-EEE virus antibody. Captured virus antigen was then detected using a 1:1000 dilution of the 1B5C-3-POD Mab and tetramethylbenzidine/H₂O₂ substrate. EEE virus antigen was successfully captured from purified virus, tissue culture seed EEE virus, sucrose acetone extracted suckling mouse brain (SMB) antigen, and artificially inoculated Aedes aegypti mosquito pools.

The antigen capture ELISA was modified for use with mosquito pools to include treatment of the supernatants with 0.5% Tween 20 in PBS to reduce background. Mosquito suspensions were considered positive if the optical density exceeded the mean optical density plus 3 standard deviations of non-infected mosquito pool controls. The results from antigen capture ELISA is compared with virus isolation from experimentally inoculated mosquitoes in Table 1.

The protocol was tested for antigen detection sensitivity with purified virus, tissue culture seed virus, SMB antigen, and antigen from virus infected mosquito pools. The overall sensitivity of this assay for EEE virus antigen in infected mosquito pools was found to be 3.5 log₁₀ of virus/0.1 ml, and was similar in antigen detection sensitivity to the SLE virus antigen capture ELISA. The specificity of the assay was tested using tissue culture seeds of western equine encephalitis (WEE) and Highlands J (HJ) viruses; only the EEE virus seed was detected. We are currently screening other EEE virus-derived Mabs for use as capturing antibodies.

References:

1. J. Clin. Microbiol. 25: 370 (1987)
2. J. Clin. Microbiol. 26: 2620 (1988)
3. Am. J. Trop. Med. Hyg. 33: 965 (1984)
4. Am. J. Trop. Med. Hyg. 33: 973 (1987)
5. J. Clin. Microbiol. 25: 976 (1987)
6. J. Amer. Mos. Control Assoc. 3: 412 (1987)
7. Am. J. Trop. Med. Hyg. 42: 394 (1990)

TABLE 1

Hours post inoculation	Sample ¹	Virus Titer ²	Capture ELISA Results	
			Experiment 1	Experiment 2
0	14A	0.7	0.102 -	0.081 -
0	14C	0.6	0.095 -	0.093 -
5.5	15A	0.4	0.095 -	0.079 -
5.5	15C	0.3	0.091 -	0.086 -
7.5	16A	0.7	0.095 -	0.079 -
7.5	16C	0.2	0.157 +	0.136 +
10.5	17A	3.1	0.105 -	0.118 -
10.5	17C	2.6	0.143 +	QNS
13.0	19A	3.5	0.123 -	0.136 +
13.0	19C	4.9	0.223 +	0.194 +
15.0	20A	3.8	0.148 +	QNS
15.0	20C	2.7	0.142 +	QNS
17.0	21A	4.0	0.169 +	0.151 +
17.0	21C	4.0	0.157 +	QNS
19.0	22A	4.7	0.259 +	0.242 +
19.0	22C	4.3	0.267 +	0.222 +
21.0	23A	5.7	0.914 +	0.751 +
21.0	23C	3.3	0.203 +	0.097 -
23.0	18A	4.7	0.384 +	0.308 +
23.0	18C	4.2	0.201 +	0.181 +
47.0	24A	4.8	0.256 +	0.234 +
47.0	24C	4.8	0.242 +	QNS
53.0	25A	5.3	0.359 +	QNS
53.0	25C	4.9	0.210 +	QNS
mean plus 3 S.D. of 16 negative mosquito pools			0.132	0.125

¹ "A" pools contain 1 infected mosquito; "C" pools contain 1 infected mosquito plus 24 uninfected mosquitoes.

² log 10 pfu/0.1ml

REPORT FROM ARBOVIRUS UNIT

Istituto Superiore di Sanità

Rome, Italy

STUDIES ON VARIABILITY OF TOSCANA VIRUS N PROTEIN

Toscana virus is a member of the *Phlebovirus* genus in the family Bunyaviridae. It belongs to the antigenic subgroup of SFN, together with SFN, Teheran and Karimabad viruses. Its natural cycle involves sand flies, bat and humans in which it causes acute Central Nervous System diseases as meningitis and meningoencephalitis.

Two major foci of this virus have been identified in Italy, one in Tuscany Region, and the other one in Marche Region.

During field studies conducted with the aim to control the distribution of the virus, we isolated more than 50 strains of TOS virus. On some representative strains we decided to initiate studies on the variability of the virus. The origin of strains included in our study are summarized in Table 1.

By IFA performed with monoclonal antibodies against N protein of the prototype ISS.Ph1.3 we evidenced some differences between TOS strains. In particular human strains seemed to be more similar than sand fly strains. To better investigate these differences we sequenced the N gene of some strains. The results are shown in Fig.1. Point mutations are present throughout the gene but the sequences are very similar as the two more distant viruses share about 97% of nucleotides. We compared the nucleotide sequences by a computer program based on the Feng and Doolittle. As shown in Fig.2 there is not relationship between the origin of the strain and the degree of similarity found in this analysis.

The predicted aminoacid sequences derived from these genes are compared in Fig.3. The majority of these mutations are silent and very few aminoacids are changed. This is not surprising as it is known that N protein is highly conserved through the *Phlebovirus* genus. N proteins are similar not only in sand fly-transmitted viruses, as TOS, PT and SFS viruses, but also in the Rift Valley Fever virus, that has different vectors, and in the tick-borne Uukuniemi virus. We can suppose that the conformation of this protein is important for its interaction with the RNA and that for this reason major changes in the aminoacid sequence have no chance to be selected.

On the basis of these results we could conclude that N genes are not good indicators for the relatedness of TOS virus strains.

L.Nicoletti, M.G.Ciufolini, C.Giorgi, S.Mochi, A.Marchi

Table.1 ORIGIN OF TOS VIRUS STRAINS

STRAIN	SOURCE	SEX	SITE	YEAR
ISS.Ph1.3	<i>Phlebotomus perniciosus</i>	F	Tuscany	1973
ISS.Ph1.32	" "	F	"	1981
ISS.Ph1.99	" <i>perfiliewi</i>	M	"	1982
ISS.Ph1.132	" "	F	"	1982
Pip.2	<i>Pipistrellus khuli</i>	?	"	1983
M.D.	Human	F	"	1983
P.I.	"	M	"	1985
P.P.	"	M	Marche	1984

Fig.1. N GENE OF TOSCANA VIRUS STRAINS

[illegible]

**Fig.2 DENDROGRAM OF GENETIC
RELATIONSHIP OF TOSCANA VIRUS STRAINS**

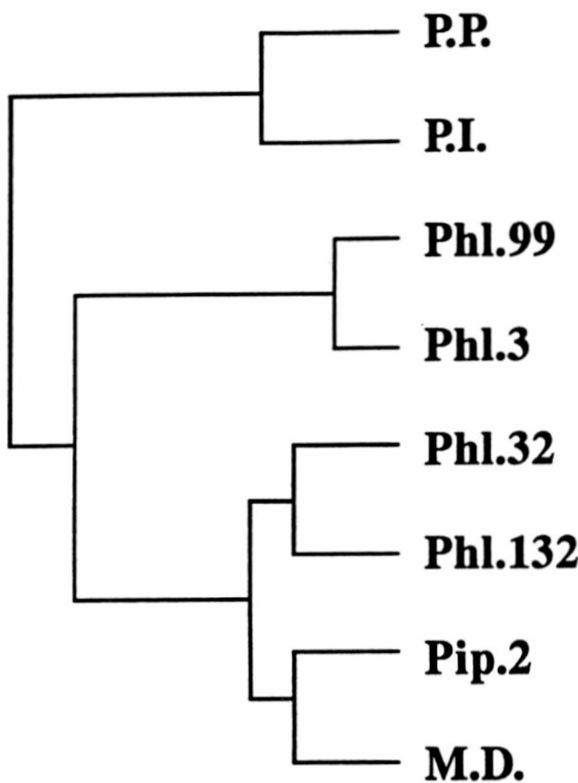


Fig.3 N PROTEIN OF TOSCANA VIRUS STRAINS

Phl. 3 Phl.99 Phl.132 Pip.2 M.D. P.I. P.P.	MSDENYRDIALAFLDESADS	GTINAWWNEFAYQGFDPKRI	VQLVKERGTAAGRWDKKDVK
	MMIVLNLVRGNKPEAMMKM	SEKGASIVANLISVYQLKEG	NPGRDTITLSRVSAAFVPWT
	VQALRVLSESLPVSGTTMDA	IAGVTYPRAMHPSFAGIID	LDLPNGAGATIADAHGLFMI
	EFSKTINPSLRTKQANEVAA	TFEKNMAAMSGRFFFTREDK	KKLLIAVGIIDEDLVLASAV
	VRSAEKYRAKVGK		

Function of Japanese Encephalitis Virus Nonstructural
Protein NS3 in RNA Synthesis.

Sequence data have suggested that Japanese encephalitis virus (JEV) protein NS3 is multifunctional, possessing virus-specific protease and helicase. To examine the functions of JEV-NS3, expression of NS3 in E. coli was carried out.

Analyses by Western blot using a monospecific anti-NS3 rabbit serum indicated that NS3 and NS3-related protein were clearly expressed, which were shown to be localized in the particle fraction of bacteria.

Pretreatment with anti-NS3 serum of the membrane fraction derived from JEV-infected cells markedly reduced the activity of RNA-synthesis in vitro by the membrane fraction. In addition, the NS3 expressed in E. coli showed RNA-binding and ATPase activities.

These results support the concept that NS3 has an important role in viral RNA replication in host cells.

TSUTOMU TAKEGAMI, Ph.D.
Associate Professor

(Corresponded by S.HOTTA)

YELLOW FEVER OUTBREAK IN MARANHÃO STATE, BRAZIL, 1993

An outbreak of yellow fever (YF) is occurring in the rural area of Mirador (45°30'W, 4°23'S) and Barra do Corda (45°16'W, 5°29'S) counties, Maranhão State, Brazil. Mirador is a very small county with a population of 20,000 inhabitants. Barra do Corda is a large county with a population of 90,000 inhabitants and is located about 100 km from Mirador.

The first death occurred in the beginning of March, the last on May 24. To date, 10 fatal cases of YF have been identified. Six were laboratory confirmed; one by virus isolation, two by serology (MACELISA), two by histopathology, and one by both serology and characteristic liver histopathology. Diagnosis of the remaining four cases was based on clinical and epidemiologic information. An additional 14 patients with YF survived; diagnosis of these was by virus isolation (two) and serology (12).

Of the 24 cases, 15 were males and 9 were females. Four were <9 years old, six were 10-19 years old, and 14 were >20 years old.

Studies are in progress to determine whether people in other counties have been affected by the virus. Ecological investigations were begun May 18. Potential vectors have been collected and will be processed for virus. More data will be available for the next issue of the Arbovirus Information Exchange.

We emphasize that the last cases of YF in Maranhão State were reported 11 years ago. There is indication that the Ministry of Health conducted a vaccination campaign against YF in that area in 1985.

Reported by: Amelia P.A. TRAVASSOS DA ROSA, Sueli G. RODRIGUES, Pedro F.C. VASCONCELOS, Nicolas DÉGALLIER*, Mário A.P. MORAES**, Jorge F.S. TRAVASSOS DA ROSA, and Ana C.R. da CRUZ

Instituto Evandro Chagas, Fundação Nacional de Saúde, Serviço de Arbovirus, Av. Almirante Barroso, 492, 66090-000, CP1128, Belém, PA, Brasil

* ORSTOM/Instituto Evandro Chagas, CP75

** Serviço de Patologia/Instituto Evandro Chagas

(Note: This report was sent by fax to the Editor, Arbovirus Information Exchange. Editor retyped the report because the copy was not clear and, in so doing, modified the text slightly but did not change the meaning.)

REPORT FROM THE VIROLOGY PROGRAM
STATE OF NEW JERSEY DEPARTMENT OF HEALTH
TRENTON, NEW JERSEY

Arbovirus Surveillance in New Jersey, 1992

During the 1992 surveillance period from June into October, 607 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were 5 mosquito pools positive for Highlands J (HJ) virus and Eastern encephalitis (EE) was isolated from 7.

Table 1 summarizes the collection area totals, species of mosquito at time of collection for the EE isolates. Activity began with late July collections and continued into October. All of the 7 isolates were from pools containing Culiseta melanura mosquitoes at 2 sites.

HJ mosquito activity is summarized in Table 2. The late September collections gave the first isolates with continued observation of HJ activity into early October. There were 5 isolates from Culiseta melanura at 3 sites.

(Shahiedy I. Shahied, Bernard F. Taylor, Wayne Pizzuti)

		Table 1 1992 EE MOSQUITO POOL ISOLATES FOR WEEK ENDING										
AREA COLLECTED	MOSQUITO SPECIES	7/31	8/7	8/14	8/21	8/28	9/4	9/11	9/18	9/25	10/2	AREA TOTALS
Dennisville	Cs. melanura	1		1				1		1	1	5
Waterford	Cs. melanura									1	1	2
WEEKLY TOTALS		1	0	1	0	0	0	1	0	2	2	7

		Table 2 1992 HJ MOSQUITO POOL ISOLATES FOR WEEK ENDING					
AREA COLLECTED	MOSQUITO SPECIES	9/18	9/25	10/2	10/9	10/16	AREA TOTALS
Bass River	Cs. melanura				1		1
Dennisville	Cs. melanura		2	1			3
Waterford	Cs. melanura		1				1
WEEKLY TOTALS		0	3	1	1	0	5

A BRIEF SUMMARY OF SURVEILLANCE FOR MOSQUITO-BORNE ENCEPHALITIS VIRUS ACTIVITY
IN CALIFORNIA DURING 1992/93

A review of the 1992 season will be published in the Proceedings and Papers of the 61st annual CMVCA conference and is also briefly summarized here.

One presumptive equine case of WEE was detected: A three year old quarter horse mare in Westmoreland, Imperial County, euthanized March 7. A single serum sample was positive for WEE at titers of 1:32 and $\geq 1:512$ by CF and IFA respectively.

Two human cases of SLE were recorded: (1) A 75-year-old woman resident of El Monte (Los Angeles County) who became ill on September 2, 1992. A blood specimen collected September 12, was tested by a private medical laboratory in Cypress, California and found to have significant titers for SLE by immunofluorescent antibody (IFA) tests. These results were confirmed by the State's VRDL by the same tests and a supplemental enzyme immunoassay (EIA) test that indicated a recent or current infection with SLE virus. The patient had not travelled outside her area of residence for at least three weeks prior to the onset of illness; (2) A 73-year-old woman resident of Oxnard (Ventura County) who became ill on September 10, 1992. Blood specimens collected 12 days and one month after illness onset and tested by the VRDL showed significant antibody titers to SLE by IFA and EIA tests. The patient had not travelled outside Ventura County during the accepted incubation period for SLE.

Tests were done by the VRDL on 2,329 mosquito pools, containing 100,613 mosquitoes, limited to the four major species most important in amplifying SLE, WEE and CE viruses. Only one virus isolate was made by the VRDL: WEE from Culex tarsalis collected in June 17, 1992 at Needles, San Bernardino County. However, the Arbovirus Research Program, School of Public Health, University of California, Berkeley, tested 1393 pools (57,597 mosquitoes) in special study areas in Imperial County and the Coachella Valley, and isolated 21 WEE and nine SLE viruses.

Sentinel chickens were located at 137 sites (including six in Nevada). Serum samples were collected and tested by enzyme immunoassay and indirect immunofluorescence for SLE and WEE antibodies twice a month from most flocks, from June through November by the VRDL, and during the winter period for selected flocks in special study areas by the U.C. Berkeley Arbovirus Research Program. Seroconversion occurred from June through November for SLE and mostly from June through August for WEE, with only two WEE seroconversions in the September-October period. A pilot study showed feasibility of doing blood collections from the comb on filter paper rather than neck vein bleeding, and this will be the routine method in 1993. Dried blood spot samples were mailed to the laboratory without refrigerant, saving time and money.

The 1993 program has started several weeks early and will be larger than in 1992, because of the extensive water abundance and mosquito breeding after years of drought conditions. Positive findings will be telephoned daily to the areas affected and weekly summaries will be sent by FAX to all State collaborative agencies.

The surveillance program involves cooperative efforts by many groups and individuals from local mosquito control agencies; the Arbovirus Research Program at the University of California at Berkeley; the California Mosquito and Vector Control Association; the CMVCA Research Foundation; county and local public health departments; the California Department of Food and Agriculture (CDFA); physicians and veterinarians throughout California; and three branches of the California Department Health Services (CDHS)-the Division of Communicable Disease Control, the Environmental Management Branch (EMB), and the Viral and Rickettsial Disease Laboratory (VRDL) of the Division of Laboratories.

Richard W. Emmons, M.D., Ph.D., Chief
Viral and Rickettsial Disease Laboratory

2

LABORATORY ACQUIRED INFECTION WITH A NEW ARENAVIRUS SP H 114.202 - CLINICAL AND LABORATORIAL FINDINGS OF A NEW HUMAN DISEASE.

Pedro Fernando da Costa VASCONCELOS¹, Amélia Paes de Andrade TRAVASSOS DA ROSA¹, Sueli Guerreiro RODRIGUES¹, Robert B. TESH², Jorge Fernando Soares TRAVASSOS DA ROSA¹ & Elizabeth Salb  TRAVASSOS DA ROSA¹.

1. Servi o de Arbovirus, Instituto Evandro Chagas, FNS/MS, Av. Almirante Barroso 492, CP 1128, 66090-000, Bel m, Par , Brasil. 2. Yale Arbovirus Research Unit, Yale University, New Haven, Connecticut, USA.

A laboratory infection with Arenavirus occurred in 39 years old male technician in August of 1992. The causative agent was a new member of the Tacaribe group (SP H 114.202) isolated in a single occasion in S o Paulo State, in 1990, from the blood of a patient with hemorrhagic fever with fatal outcome (LISIEUX et al, 1993, XXIX Congresso da Sociedade Brasileira de Medicina Tropical, programa e resumos, p. 331).

The patient, became ill for 13 days. The disease had an abrupt onset characterized by high fever (39 C.), headache, chills and myalgias for 8 days. On the other hand, in the 3rd day, the patient developed nausea and vomiting, and in the 10th, epigastric pain, diarrhea and gingival bleeding. Leukopenia (2,500 WBC) was observed in the 1st week of illness. Counts performed after the 23th day of illness were within normal limits. With the exception of moderate lymphocytosis, no changes were observed in differential counts. Transaminases (SGOT and SGPT) had a transitory increase.

Suckling mice and baby hamsters were inoculated intracerebrally with 0.02 ml of a blood sample collected in the 2nd day of disease. Attempts to virus isolation were also made into Vero cells. No virus was isolated.

Serum samples taken the patient at different times showed the following results by CF, Neutralization and MAC ELISA tests.

D A T E	DAYS AFTER ONSET	T I T L E R		
		C F	N T *	MAC ELISA **
September, 1	3	<8	<1.2	ND
" " 8	10	<8	<1.4	0.097
" " 14	16	<8	2.6	0.430
" " 21	23	8	2.4	0.411
October, 7	39	>64	ND	0.234
" " 27	59	32	3.4	0.158
November, 12	75	32	ND	ND

* Expressed in LD₅₀

** Optical density. Values >0.2 are positives.

The mode of infection is unknown although infectious aerosols are suspected. The patient denied contact with the infecting agent during three weeks prior to onset of disease and he reported no cuts or abrasions while working with it.

Clinical features of haemorrhagic fever with renale syndrome in Slovakia

O. Kožuch¹, A. Baráková², M. Labuda¹

¹WHO Collaborating Centre for Arbovirus Reference and Research, Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, ²National Institute of Hygiene and Epidemiology, 826 45 Bratislava, Slovak Republic

In the period of 1989-1991 12 clinically diagnosed cases of haemorrhagic fever with renale syndrome (HFRS) were serologically confirmed among the sera of 75 patients with suspected diagnose of HFRS. By the method of indirect immunofluorescence the antibodies to Hantaan and Puumala viruses (genus *Hantavirus*) were found in 6 patients each. Antibody titres varied from 1:16 to 1:1024. From 12 confirmed HFRS patients 11 had mild form of disease (5 with antibodies to Hantaan virus and 6 with antibodies to Puumala virus) and one patient had severe form of HFRS (antibodies to Hantaan virus) with wide range of clinical symptoms (Table 1).

Patients were 10 males and two females, 17 - 56 years of age, 6 professionals in forestry and agriculture and the other 6 also visiting field and forest frequently. Eleven patients indicated direct or indirect contacts with rodents in the incubation period which was 4 - 42 days. Geographically, 7 cases were from eastern Slovakia, 1 case from central Slovakia and 4 cases from western Slovakia.

Previously, in accordance with findings of others, the importance of rodents in the epidemiology of the disease in Slovakia has been documented. Antigen of Puumala virus was demonstrated in the lung tissues of *Clethrionomys glareolus* and *Microtus arvalis*; antigen of Hantaan virus was detected in *Apodemus agrarius*, *A. flavicollis* and *M. arvalis* (suspected different Hanta-like serotype from *M. arvalis*). In these rodent species also antibodies to both hantaviruses were detected in 8% (Grešíková et al. 1986, Acta virol. 30: 158-160).

Table 1.

Clinical picture of HFRS in Slovakia

Form of disease	mild		severe
Virus serotype	Puumala	Hantaan	Hantaan
Symptoms			
Fever	6	5	1
Somnolence	-	-	1
Headache	5	2	1
Meningism	3	1	1
Backache	1	2	-
Abdominal pains	3	1	-
Nausea	2	1	1
Petechiae	-	1	-
Flush over the face	1	-	-
Red throat	3	2	1
Diarrhea	3	1	1
Conjunctival injection	2	2	1
Visual disturbances	1	1	-
Cough	3	3	-
Hypotension	-	1	-
Hypertension	-	2	-
Hematuria	2	5	-
Proteinuria	4	5	1
Creatinine (over 115) ⁺	-	5	1
Urea (over 7.0) ⁺⁺	-	3	1
Acute renal insufficiency	2	3	1
Brain edema	-	-	1
No. of patients	6	5	1

⁺Creatinine elevated (115 - 1273 mmol/l)

⁺⁺Serum urea nitrogen elevated (7.1 - 46.6 mmol/l)

ICTERUS AND ARF : LEPTOSPIROSIS OR HANTAVIRUS DISEASE ?

J. Clement¹, P. McKenna¹, P. Matthys¹, C. McCaughey², G. Neild³, S. Hindrichsen⁴, H. Leirs⁵, G. van der Groen⁶.

1. J. Clement, P. McKenna & P. Matthys : Belgian Zoonosis Workgroup, Queen Astrid Military Hospital, Bruynstraat, B-1120 Brussels, Belgium.

2. C. McCaughey : Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BN, N-Ireland.

3. G. Neild : The Middlesex Hospital, Mortimer Street, W1N 8AA London, UK.

4. S. Hindrichsen : Oswaldo Cruz Hospital, Federal University of Pernambuco, Recife, P.E. 52061 Brazil.

5. H. Leirs : General Zoology Laboratory, State University Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp, Belgium.

6. G. van der Groen : Microbiology Laboratory, Institute of Tropical Medicine (ITM), Nationalestraat 155, B-2000 Antwerp, Belgium.

Correspondence address

Dr. J. Clement : Belgian Zoonosis Workgroup (CTISM)
Queen Astrid Military Hospital
Bruynstraat, B-1120 Brussels, Belgium
Tel +32/2/268.00.50 Ext 2464 - 2442
Fax +32/2/268.72.49

SUMMARY

Rat-transmitted strains of *Hantavirus* (HV), can cause infectious forms of ARF which closely mimic Leptospirosis (LSP). In a Belgian serosurvey, the IFA IgG Hantaviral antibody prevalence of 2.1% (44/2,055) in sera suspected of LSP, was significantly higher than the 1.3% prevalence (129/9,413) in a reference group of healthy blood donors ($\chi^2 = 10.52$ $p < 0.001$).

We found serological evidence of Hantavirus disease (HVD) in 8 out of 156 (5.1%) Brazilian cases suspected of LSP, and in 2 recent Irish cases presenting with ARF and jaundice, suggestive of LSP. So far, HVD had not been reported in either country. All of these 10 patients had diagnostic IFA Ig titers against the Chinese Hantaviral strain R22, but were negative in *Leptospira* ELISA, except one Brazilian patient which was positive for both. Moreover, sera reacted in IFA only to the rat-transmitted strain R22 and negatively or only atypically to the "classical" HV-strains, *Puumala* (PUU) and *Hantaan* (HTN), carried by other rodents. μ -capture IgM ELISA, using both PUU and HTN, could pick up only 2/10 cases.

In conclusion : A rat-derived Hantaviral strain such as R22 should be included in the screening of ARF presenting as LSP.

KEY WORDS

Acute renal failure (ARF), Hantavirus, Icterus, Leptospirosis, Rat, Zoonosis.

RUNNING TITLE

ICTERUS AND ARF : LEPTOSPIROSIS OR HANTAVIRUS DISEASE ?

Hantavirus disease (HVD) is a newly recognized zoonosis consisting of acute febrile illness (1), most often complicated by acute renal failure (ARF), thrombocytopenia and hemorrhagic manifestations. The etiologic agent is a rodent-borne hemorrhagic fever virus called *Hantavirus*, a new genus in the Bunyaviridae family, of which at least 6 clinically important serotypes have been isolated worldwide (2), each carried by a specific rodent vector. The laboratory rat has been the source of outbreaks of human HVD among laboratory personnel worldwide (3,4). So far, the wild rat (*Rattus norvegicus*) has been incriminated as an important vector only in the Far East, where HVD due to the *Seoul* (SEOU) serotype has been documented in urban (5) and in rural outbreaks (6). Whereas liver pathology in the *Puumala* (PUU)-induced European forms seemed always mild and limited to a transient elevation of serum transaminases, hepatitis-like forms with or without icterus have been noted in the *Seoul* (SEOU)-induced forms in the Far-East, in some instances even without renal involvement (7). We present here the first (to our knowledge) non-laboratory *Seoul* serotype cases of HVD on the American (Brazil) and on the European (N-Ireland) continent, in whom the clinical presentation evoked in the first place the diagnosis of leptospirosis (LSP).

SUBJECTS AND METHODS

A/ SUBJECTS

1. Belgian serosurvey

Up to January 1986, screening for the presence of specific *Hantaan* 76-118 (HTN) IgG antibodies was performed at the Institute of Tropical Medicine, Antwerp (Belgium) in a total of 9,413 healthy asymptomatic Belgian blood donors and in a total of 2,055 sera submitted from all over Belgium to exclude leptospirosis.

2. Brazilian patients

156 sera were collected in 1990 from 144 patients admitted to the Cruz Hospital, Federal University of Pernambuco, Recife (NE Brazil) with a clinical diagnosis of acute leptospirosis (8).

3. Irish patients

During the 1989-1992 period, a total of 626 serum samples were examined in the Royal Victoria Hospital, Belfast (N-Ireland), from patients presenting with symptoms compatible with acute HVD (9).

B/ METHODS

1. Hantaviral serology

Indirect Immunofluorescence antibody assay (IFA) : VERO E6 cells infected with the different Hantaviral strains were challenged with patients' sera from a dilution of 1/16 on and subsequently marked with goat anti-human IgG*.

μ -capture ELISA : A commercially available assay (Progen^R, Heidelberg) was used, incorporating HTN and PUU recombinant nucleoprotein antigens.

2. Leptospiral serology

A home-made ELISA-test using microtiter plates coated with a thermostable group antigen Biflexa-Patoc (Courtesy of Dr Bigaignon, Microbiology, University Hospital St Luc (UCL), Brussels, Belgium) was used.

3. Statistical analysis

For comparing study groups, chi square and Fisher exact test were used.

RESULTS

1. Belgian serosurvey

We found in the leptospirosis-suspected patient sera an anti-*Hantaan* (HTN) seroprevalence of 2.1% (44/2,055) vs 1.3% (124/9,413) in the reference blood donor group.

2. Brazilian patients & Irish patients

See Table I.

Of the 156 Brazilian LSP-suspected sera, 8 (5.1%) were found to possess specific IFA IgG antibodies against R22 strain transmitted by the rat, but were negative or gave only atypical immunofluorescence against the other Hantaviral strains (8). Of the 626 Irish sera, 16 (2.5%) appeared IgG-positive against the R22-strain (9). Of these 16 seropositives, 2 patients (12.5%) presented with fever, jaundice and acute renal failure, but were found to be ELISA-negative for LSP.

DISCUSSION

We found significantly higher ($X^2 = 10.52$, $p < 0.001$) Hantaviral seroprevalence in Belgian LSP-suspected sera (2.1%) compared with the reference blood donors' sera (1.3%), suggesting at least that a clinical diagnosis of LSP is often put forward in cases where in fact Hantavirus infections did occur. The wild rat as a common vector worldwide for both pathogens, is further complicating the picture since apparently dual infections have been described (10,11). LSP is a common zoonosis in Brazil, where up to 78% of the rats captured were shown to be infected with LSP (12). However in 190 of the cases of LSP reported in 1986 in the State of Sao Paulo, 31 (16.3%) remained seronegative, despite the fact that up to 11 different serovars of the pathogenic species *Leptospira interrogans* were used for screening. Serologically proven Hantavirus disease had never been reported so far from Brazil (nor from the rest of the American continent) despite the fact that LeDuc et al isolated a HNT 76-118-related Hantavirus from Brazilian rats in 1985, and demonstrated the widespread distribution of Hantavirus in South-America (13). In our recently reported Hantaviral R22-positive Brazilian cases (8), we found no evidence in ELISA for leptospiral antibodies, except in one case [n°6] that was seropositive for both pathogens HTN and LSP.

It is noteworthy that without the rat-strain R22, our cases in Brazil and in N-Ireland could easily have been missed in conventional Hantavirus serology, using the two "classical" Hantaviral strains HTN 76-118 and PUU (Table I). μ -capture ELISA IgM assays are usually advocated for seroconfirmation of recent HVD-cases (14). The fact that this new and sensitive assay failed to pick up 8/10 of the herein described cases is probably due to the fact that the rat derived R22 was not incorporated as a screening antigen.

A rat-derived Hantaviral strain such as R22 should be included in serological screening of ARF presenting as LSP.

REFERENCES

1. Clement J, van der Groen G. Acute Hantavirus nephropathy in Belgium : Preliminary results of a sero-epidemiological study. In : Acute Renal Failure. (Eds. Amerio A., Coratelli P. et al), New York, Plenum publishing corporation; 1987;251-263.
2. Xiao S-Y, Chu Y-K, Knauert K, Loftis R, Dalrymple JM, LeDuc JW. Comparison of Hantavirus isolates using a genus-reactive primer pair polymerase chain reaction. J Gen Virol 1992;73:567-573.
3. Desmyter J, Johnson KM, Deckers C, LeDuc JW, Brasseur F, van Ypersele de Strihou C. Laboratory rat associated outbreak of hemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. Lancet 1983;ii:1445-8.
4. Yanagihara R, Gajdusek DC. HFRS : a historical perspective and review of recent advances. In : Handbook of viral and rickettsial haemorrhagic fevers. (Gear JHS, ed), Boca Raton, Florida. CRC Press. 1988;155-181.
5. Lee HW, Park DH, Baek LJ, Choi KS, Wang YN, Woo MS. Korean hemorrhagic fever patients in urban areas of Seoul. Korean J Virol 1980;10:1-6.
6. Song G, Hang C, Liao H, et al. Isolation of EHF related agent from Rattus norvegicus captured from patients' home in endemic area of the mild type of hemorrhagic fever. Acta Microbiol Sin 1982;22:373-7.
7. Wong TW, Chan YC, Joo YG, Lee HW, Lee PW, Yanagihara R. Hantavirus infections in humans and commensal rodents in Singapore. Trans R Soc Trop Med Hyg 1989;83(2):248-51.
8. Hindrichsen S, Medeiros de Andrade A, Clement J, Leirs H, McKenna P, Matthys P. Hantavirus infection in Brazilian patients from Recife with suspected leptospirosis. Lancet 1992;341:50.
9. McKenna P, Clement J, Matthys P, Coyle PV, McCaughey C. Serological evidence of Hantavirus disease in Northern Ireland. (submitted for publication to J Med Virol).
10. Kudesia G, Christie P, Walker E, Pinkerton J, Lloyd G. Dual infection with leptospira and Hantavirus. Lancet 1988;i:1397.
11. Davies EA, Rooney PJ, Coyle PV, Simpson DI, Montgomery IW, Stanford CF. Hantavirus and Leptospira. Lancet 1988;ii:460-1.
12. Correa MOA, Veronesi R, De Brito T, Hyakutake S, Santa Rosa CA, Edelweiss EL. Leptospirosis. In : (Veronesi R, ed) Doenças Infecciosas e Parasitárias. Rio de Janeiro, Editora Guanabara Koogan SA. 1982;573-92.
13. LeDuc JW, Smith GA, Pinheiro FP, Vasconcelos PFC, Rosa EST, Maiztégui JI. Isolation of a Hantaan-related virus from Brazilian rats and serologic evidence of its widespread distribution in South-America. Am J Trop Med Hyg 1985;34:810-5.
14. Niklasson B, Kjelsson T. Detection of Nephropathia epidemica (Puumala) virus specific immunoglobulin M by enzyme linked immunosorbent assay. J Clin Microbiol 1988;26:1519-23.

TABLE I : SEROLOGICAL DATA IN 10 PATIENTS SUSPECTED OF LSP

Patient N°	HTN 76-118	CG 18-20	CG 13891	TCH	R22 VP30	p-capture PUU	EIA HTN	EIA Lepto
- BRAZILIAN PATIENTS								
1	A	A	NT	NT	64*	neg	neg	neg
2	A	A	NT	NT	32	neg	neg	neg
3	A	A	NT	64	0.714	neg	neg	
4	A	A	NT	NT	128	neg	neg	neg
5	A	A	NT	NT	64	0.454	0.384	neg
6	A	A	NT	NT	64	neg	neg	pos
7	A	A	NT	NT	128	neg	neg	neg
8	A	A	NT	NT	32	neg	neg	neg

- IRISH PATIENTS

1	neg	neg	neg	neg	256	neg	neg	neg
2	neg	neg	neg	neg	64	neg	neg	neg

* = reciprocal of the highest dilution still positive in IFA.

NT = NOT TESTED - A = Atypical fluorescence pattern

Because so many asked what I was doing in Australia for four months, and some further asked me to summarize the trip in this issue of the Arbovirus Information Exchange, I am doing so. In summary, I can honestly say that it was the best four months of my life. First and foremost, one cannot speak of Australia without mentioning the remarkable flora and fauna, including the Aussies themselves. They seem to be making as big a mess of their portion of the world as the rest of us are with ours; however, there is enough of the original for a tourist to be entranced.

I spent the first three weeks with Professor Adrian Gibbs at Australian National University, Canberra, learning the Australian language and trying to learn something of the DELTA system so that I could establish a data base for arboviruses. Not being much of a computer person, it took me weeks simply to figure out what the terminology meant; Adrian speaks neither Australian nor American. However, given some long days and nights, and with his gentle prodding and encouragement, I was successful. That is to say, we now have the rudiments of a data base and, should the ICTV one day find funding, we can integrate the arboviruses with all the other viruses of the world. There are hundreds more viruses to enter and I did not begin to enter the arboviruses of veterinary importance, but we did make a grand beginning.

Next, I spent a month with Lorna Melville, Neville Hunt, and Richard Weir, at the Agricultural Research Centre, Berrimah, outside Darwin in the Northern Territory. Richard and I began to sort out and identify the hundreds of viruses (from mosquitoes) not previously identified. Geoff Gard and others had been looking for certain viruses and, if they did not identify an isolate as one of them, they put it back in the freezer for another time; this was the other time. Using IFA, we made some headway and Richard is continuing to identify these isolates as the time is available to do so. He is collaborating with Alex Hyatt (Australian Animal Health Laboratories, Geelong) in this work. To approximate date, Richard has provisionally identified 27 isolates of Wongorr, 3 Wallal, 4 Warrego, 3 Eubenangee, 4 Corriparta, 2 Mapputta, 3 Mapputta serogroup, 4 Kowanyama, 1 Trubanaman, 1 rhabdovirus, 10 alphavirus type I, 13 alphavirus type II, and 3 flaviviruses. The type I and type II alphaviruses are different from each other but, because this has all been done by IFA, none of these results can be considered anything but provisional and very preliminary.

All work and no play making Charlie a dull boy, I spent a few days touring the remarkable Kakadu National Park, seeing as much as I could of that huge place and learning as much as I could about the wildlife there, particularly the crocodiles, with whom I have much in common and which are the most fascinating animals I have come across. Call me sometime (not collect) and I will tell you about them. The hundreds of species of magnificent birds, the water buffalo, dingos, hundreds of species of the genera *Eucalyptus* and *Acacia*, and the Australian in his and her natural habitat were among the many species of wildlife I enjoyed. I did not enjoy the flies.

Then to Perth, which is at the edge of the world, and the University of Western Australia. Professor John Mackenzie and his staff were superb hosts and I even had the opportunity to help work out an IgM capture ELISA for some of the viruses in which they are interested. As you know from previous issues of the AIE, this is a very active group, working to understand the epidemiology, field biology, virology, and molecular biology of Murray Valley encephalitis, Ross River, and other arboviruses. I drove to the far southwest corner of Australia and toured the coast and the fantastic (the only adequate word I know) forests. There are many bars in Australia.

From Perth I flew to Melbourne and spent a week with Tony Della-Porta and co-workers at AAHL in Geelong. Mostly we were comparing notes and discussing the establishment of the arbovirus data base, in which Tony has considerable interest. I tried as many as I could of the great wines of South Australia. I only made a dent, so I must return.

Then to Brisbane for a week at the arbovirus symposium, presenting a couple of papers, renewing acquaintances, testing the local beers, and doing my share of aggravating Toby St. George (who pretends to be retired).

I returned to Canberra and went with the Gibbs family to the stark and lovely coast of New South Wales. We didn't do much other than see the sights, watch the birds, and look for evidence of plant viruses. Adrian is a remarkable biologist and human being and plant viruses are as fascinating as other viruses, but there is only so much my brain will absorb in one 4-month period.

Shelley flew over for winter break from teaching and we spent three weeks together: first on Heron Island on the Great Barrier Reef snorkeling, eating good food, and watching Green Sea Turtles (*Chelonia mydas*) and Loggerhead Turtles (*Caretta caretta*) lay eggs; then driving north along the coast seeing the sights, drinking beer, looking for crocodiles, and wandering through primeval rainforests (no room here to expound). Finally, to the Atherton Tableland, where we mostly rested, watched birds, and looked at the scenery.

When Shelley returned home I went to Sydney and spent more than a week with Linda Hueston and Richard Russell at Westmead Hospital, working out a technique that would allow Linda to use a "cocktail" of antigens for an IgM capture ELISA for antibodies to flaviviruses. We also took in some sights and tried some of the local wines.

Finally, I returned to Australian National University and attended a symposium "Frontiers in Molecular Evolution", organized by Adrian. Then I came home.

Had it not been for the extraordinary (really!) hospitality and many kindnesses of Adrian Gibbs, Ian Marshall, Frank Fenner, Geoff Gard, Lorna Melville, Neville Hunt, Richard Weir, John Mackenzie, Annette Broom, Bob Coelen, Tony Della-Porta, Toby St. George, Daisy Cybinski, Peter Egler (Heron Island), Linda Hueston, Richard Russell, Mike Cloonan, and their families and so many others who fed and housed me, provided me with a bit of spending money here and there, took the time and effort to transport me hither and yon, and otherwise were uncommon hosts, my stay might have been filled with days at dusty museums and art galleries. Better than that, my days were spent in laboratories or amidst beautiful scenery and beautiful people, and my nights were spent in bars. Should you be able to escape your present life, I suggest Australia.

As you might guess, I am looking for a job. This retirement is killing me.

Charles H. Calisher, Ph.D. (Arthropod-borne Infectious Disease Laboratory, Colorado State University, Ft. Collins, Colorado)

QUOTES

Mickey Mantle: "The question is not whether the pitcher is a son of a bitch but whether the son of a bitch can pitch."

Lewis Grizzard: "I'm not going to get married again. I'm just going to find a woman I hate and buy her a house."

Isaac Bashevis Singer: "My favorite readers are the very young because children read books not reviews. Besides, they still believe in God, the family, angels, devils, witches, goblins, logic, clarity, punctuation and other such obsolete stuff."

Marion Smith: "The trouble with the 1980's, as compared with the 1970's, is that teenagers no longer rebel and leave home."

Herb Caen: "The only thing wrong with immortality is that it tends to go on forever."

George Miller: "The trouble with eating Italian food is that five or six days later you are hungry again."

Lucretius: "Some species flourish, others are diminished- and soon the generations of living creatures are changed, and like runners relay the torch of life."

E.O. Wilson: "The present era is the sixth mass extinction event in history."

E.O. Wilson: "Fund taxonomy!"

Fred Allen: "The town was so dull that when the tide went out it refused to come back."

Steven Pearl: "I cannot believe that out of 100,000 sperm, you were the quickest."

Samuel Johnson: "One of the disadvantages of wine is that it makes a man mistake words for thoughts."

Dave Barry: "The Japanese may be ahead of us in some areas of technology, but they're a long way from being able to produce, for example, James Brown."

Albert Einstein: "I want to know how God created this world. I am not interested in this or that phenomenon, in the spectrum of this or that element; I want to know His thoughts; the rest are details."

In Memory of Jelka-Vekenjak

Edmund Burke: "The only thing necessary for the triumph of evil is for good men to do nothing."